

## CHEMICAL CONSTITUENTS FROM STEM BARKS AND ROOTS OF *MURRAYA KOENIGII* (RUTACEAE)

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### Abstract

Four carbazole alkaloids, identified as mahanimbine (1), girinimbine (2), murrayanine (3) murrayafoline-A (4) and one triterpene were isolated from stem bark and roots of *Muraya koenigii*. The structures of these compounds were established by infra-red (IR), mass spectrometry (MS) and nuclear magnetic resonance (<sup>1</sup>H NMR, <sup>13</sup>C NMR, HMQC and HMBC) spectroscopy.

### Abstrak

Empat alkaloid karbazol, dikenali sebagai mahanimbine (1), girinimbine (2), murrayanine (3), murrayafoline-A (4) dan satu triterpen telah dipencilkan daripada batang dan akar *Muraya koenigii*. Struktur-struktur bagi semua sebatian kimia dibuktikan dengan kaedah spektroskopi inframerah (IR), spektroskopi jisim (MS), dan spektroskopi resonans magnet nukleus (<sup>1</sup>H NMR, <sup>13</sup>C NMR, HMQC and HMBC).

### Introduction

*M. koenigii* (Rutaceae) is one of the two species of *Muraya* found in Peninsular Malaysia. The plant usually cultivated for its aromatic leaves is normally used for natural flavoring in curries and sauces [1]. This plant is also distributed in India, Andaman Islands and throughout Central and Southeast Asia [7]. The plant was spread to Malaysia, South Africa and Reunion Island by South Asian immigrants. Parts of the plant have been used as raw material for traditional medicine formulation in India [4]. *M. koenigii* leaves and roots can be used to cure piles and allay heat of the body, thirst, inflammation and itching. *M. koenigii* is known to be the richest source of carbazole alkaloids. It has been reported by previous that carbazole alkaloids possess various biological activities such as anti-tumor, anti-oxidative, anti-mutagenic and anti-inflammatory activities [5, 6]. In 1978, Chakraborty *et al.*, [2] isolated mukonine, a carbazole alkaloids from the stem bark of *M. koenigii*. In continuation of our investigation on Rutaceous plants, research on *M. koenigii* was carried out. Phytochemical studies on this plant species collected from Northern Peninsular Malaysia has been reported previously [5].

### Experimental

#### Material and methods

The stem bark and roots of *M. koenigii* has been used in this study were collected from Sik, Kedah in 2005. Air dried plant material (about 1 kg) was ground into fine powder and extracted successively with hexane, chloroform and methanol. The solvent was removed using rotary evaporator and each of the extract was subjected to column chromatography separation. The column was eluted with mixtures of hexane, chloroform and methanol with 10% increment of each mixture. Work-up procedure on the fractions have afforded five carbazole alkaloids (1- 4), and one triterpene.

#### Plant Material

The stem bark and roots of *M. koenigii* used in this study was collected from Sik, Kedah in December 2005.

#### Instrumentation

Melting points (uncorrected) were determined on Kohfler melting points apparatus. The IR spectra were recorded using KBr disc on Perkin Elmer FTIR spectrophotometer model 1725X. <sup>1</sup>H and <sup>13</sup>C NMR spectra were obtained on JOEL Spectrometer at 400 and 100 MHz, respectively with tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on Shimadzu model QP5050A at 70 eV. Column chromatography was

carried using silica gel (Merck 7749) and Merck silica gel 60 PF<sub>254</sub> was used for Thin Layer Chromatography (TLC) analysis.

#### Extraction and Isolation

Extractions and separations on the isolates of hexane, chloroform and methanol of the plant samples (stem bark and roots) have led to the isolation and characterizations of carbazole alkaloids. The compounds were elucidated using spectroscopic methods (IR, 2D-NMR and MS). Stem barks and roots of *M. koenigii* extracts was concentrated under reduced pressure to yield a brown yellowish viscous syrup for crude hexane extract (22.5 g and 33.0 g) and dark brown viscous syrup for crude chloroform extract (14.0 g and 24.0 g). Each crude extracts was subjected to column vacuum chromatography over silica gel and eluted with mixture of hexane, hexane/ ethyl acetate, ethyl acetate, ethyl acetate/ methanol and methanol to give a total about 75 fractions each.

Fractions A9-A18 (crude hexane extract of stem barks) (eluent: hexane/ ethyl acetate/ methanol) were combined to give mahanimbine **1** (4.2 g): white solid, C<sub>23</sub>H<sub>25</sub>NO, m.p. 88-90 °C (lit. 94-95 °C [3]). IR  $\nu_{\max}$  (cm<sup>-1</sup>, KBr disc): 3324 (N-H), 2924, 1646 (C=C), 1610, 1458, 1378, 1332 (C-N), 1218 (C-O), 746, 680. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.90 (1H, *d*, *J*=8.28 Hz, H-5), 7.87 (1H, *br*, N-H), 7.66 (1H, *s*, H-4), 7.19 (1H, *t*, *J*=7.36 Hz, H-6), 7.37 (1H, *d*, *J*=8.28 Hz, H-8), 7.31 (1H, *t*, *J*=7.32 Hz, H-7), 6.65 (1H, *d*, *J*=9.16 Hz, H-9), 5.67 (1H, *d*, *J*=9.16 Hz, H-10), 5.13 (1H, *t*, *J*=7.36 Hz, H-3'), 2.33 (3H, *s*, 13-Me), 2.17 (2H, *q*, 2'-CH<sub>2</sub>), 1.78 (2H, *t*, *J*=8.24 Hz, 1'-CH<sub>2</sub>), 1.66 (3H, *s*, 6'-Me), 1.58 (3H, *s*, 12-Me), 1.45 (3H, *s*, 5'-Me). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  149.9 (C-2), 139.4 (C-1a), 134.9 (C-8a), 131.7 (C-4'), 128.5 (C-10), 124.2 (C-7), 123.9 (C-3), 121.2 (C-4), 119.3 (C-5), 119.1 (C-6), 118.6 (C-5a), 118.4 (C-3'), 117.5 (C-9), 116.6 (C-4a), 110.4 (C-8), 104.2 (C-1), 78.1 (C-11), 40.7 (C-1'), 25.8 (C-5'), 22.7 (C-2'), 17.6 (C-12), 16.1 (C-13). MS (*m/z*, % intensity): *m/z* 331 (M<sup>+</sup>, 17), 316 (4), 248 (100), 218 (5), 204 (8), 69 (4), 55 (4).

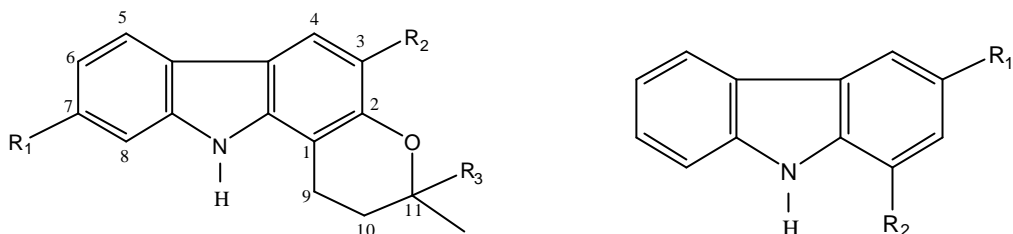
Fractions B9-B12 (crude hexane extract of roots) (eluent: hexane/ ethyl acetate/ methanol) were combined to give girinimbine **2** (1.0 g): white crystals, C<sub>18</sub>H<sub>17</sub>NO, m.p. 175-177 °C (lit. 177-178 °C [3]). IR  $\nu_{\max}$  (cm<sup>-1</sup>, KBr disc): 3316 (N-H), 2974, 2930, 1640 (C=C), 1606, 1492, 1360 (C-N), 1344, 1318 (C-O), 1206, 924, 784, 688. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  7.92 (1H, *d*, *J*=8.28 Hz, H-5), 7.84 (1H, *br*, N-H), 7.66 (1H, *s*, H-4), 7.36 (1H, *d*, *J*=8.28 Hz, H-8), 7.31 (1H, *t*, *J*=8.24 Hz, H-7), 7.19 (1H, *t*, *J*=8.82 Hz, H-6), 6.60 (1H, *d*, *J*=9.20 Hz, H-9), 5.69 (1H, *d*, *J*=9.20 Hz, H-10), 2.33 (3H, *s*, 14-Me), 1.56 (3H, *s*, 12-Me), 1.48 (3H, *s*, 13-Me). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  149.8 (C-2), 139.4 (C-1a), 134.8 (C-8a), 129.4 (C-10), 124.2 (C-7), 123.9 (C-3), 121.1 (C-4), 119.4 (C-6), 119.3 (C-5), 118.6 (C-5a), 117.2 (C-9), 116.7 (C-4a), 110.4 (C-8), 104.4 (C-1), 75.8 (C-11), 27.6 (C-12), 27.6 (C-13), 16.1 (C-14). MS (*m/z*, % intensity): *m/z* 263 (M<sup>+</sup>, 69), 248 (100), 231 (8), 218 (10), 204 (17), 191 (5), 131 (5), 124 (66), 102 (16), 95 (7), 82 (5).

Fractions B24-B27 (crude hexane extract of roots) (eluent: hexane/ ethyl acetate/ methanol) were combined to give murrayanine **3** (0.5 g): white needle-shaped crystals, C<sub>14</sub>H<sub>11</sub>NO<sub>2</sub>, m.p. 165-167 °C (lit. 168-169 °C [3]). IR  $\nu_{\max}$  (cm<sup>-1</sup>, KBr disc): 3150 (N-H), 1662 (C=O), 1608 (C=C), 1578, 1500, 1450 (C-N), 1342, 1138 (C-O), 848, 822, 608. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  10.06 (1H, *s*, CHO), 8.61 (1H, *br*, N-H), 8.20 (1H, *s*, H-4), 8.13 (1H, *d*, *J*=7.32 Hz, H-5), 7.53 (1H, *t*, *J*=8.24 Hz, H-7), 7.50 (1H, *d*, *J*=6.44 Hz, H-8), 7.47 (1H, *s*, H-2), 7.34 (1H, *t*, *J*=8.24 Hz, H-6), 4.08 (3H, *s*, O-Me). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  191.9 (3-CHO), 146.1 (C-1), 139.4 (C-1a), 134.1 (C-8a), 130.16 (C-3), 126.6 (C-8), 123.64 (C-4a), 123.64 (C-5a), 120.69 (C-5), 120.69 (C-6), 120.41 (C-4), 111.47 (C-7), 103.5 (C-2), 55.8 (1-OMe). MS (*m/z*, % intensity): *m/z* 225 (M<sup>+</sup>, 100), 210 (67), 196 (7), 182 (22), 181 (11), 154 (61), 153 (21), 126 (28), 112 (7), 98 (14), 87 (10), 63 (8), 51 (21).

Fraction D10 (crude chloroform extract of roots) (eluent: hexane/ ethyl acetate/ methanol) to give murrayafoline-A **4** (0.3 g): yellowish needle-shaped crystals, C<sub>14</sub>H<sub>13</sub>NO, m.p. 50-52 °C (lit. low melting point [3]). IR  $\nu_{\max}$  (cm<sup>-1</sup>, KBr disc): 3448(N-H), 2996, 2942, 2916, 2840, 1924, 1694, 1590(C=C), 1502, 1388, 1332 (C-N), 1304, 1278, 1130, 1104 (C-O), 1036, 942, 852, 742, 670. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>):  $\delta$  8.14 (1H, *br*, N-H), 8.01 (1H, *d*, *J*=7.32 Hz, H-5), 7.47 (1H, *s*, H-4), 7.37 (1H, *d*, *J*=8.28 Hz, H-8), 7.20 (1H, *t*, *J*=1.84 Hz, H-7), 7.19 (1H, *t*, *J*=1.84 Hz, H-6), 6.72 (1H, *s*, H-2), 3.97 (3H, *s*, O-Me), 2.52 (1H, *s*, H-9). <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>):  $\delta$  145.3 (C-1), 139.4 (C-8a), 129.4 (C-4a), 127.9 (C-3), 125.5 (C-8), 124.3 (C-5a), 123.5 (C-1a), 120.4 (C-6), 119.1 (C-5), 112.5 (C-4), 110.9 (C-7), 107.6 (C-2), 55.4 (1-OMe), 21.9 (C-9). MS (*m/z*, % intensity): *m/z* 211 (M<sup>+</sup>, 100), 196 (73), 182 (3), 168 (43), 167 (36), 139 (5), 106 (9), 84 (6).

### Results and Discussion

The stem bark and roots of *M. koenigii* were extracted with hexane, chloroform and methanol. The fractionation of the hexane and chloroform extracts followed by column chromatography and TLC yield carbazole alkaloids which were identified as mahanimbine (1), girinimbine (2), murrayanine (3) and murrayafoline-A (4) by spectroscopic methods and direct comparison of spectral data to those published in the literature. 2D NMR study including HMQC, HMBC and COSY correlation techniques was undertaken on murrayafoline-A (4) to complement the reported NMR data.



- (1) : Mahanimbine;  $R_1=H$ ,  $R_2=CH_3$ ,  $R_3=-(CH_2)_2CH=C(CH_3)_2$  (3) : Murrayanine;  $R_1=CHO$ ,  $R_2=OCH_3$   
 (2) : Girinimbine;  $R_1=H$ ,  $R_2=R_3=CH_3$  (4) : Murrayafoline-A ;  $R_1=CH_3$ ,  $R_2=OCH_3$

Mahanimbine (1) was obtained as white powder from fractions A9-A18 of column chromatography of hexane extract, eluted with 5% ethyl acetate in hexane. The solid was recrystallized with hexane to give white solid (4.2 g) that had a melting point of 88-90 °C, lit. m.p. = 92-94 °C [3]. Purification of fraction B10-B12 obtained from column chromatography of the hexane extract of the roots, followed by recrystallization in ethyl acetate yielded girinimbine (2) (1.0 g), m.p. 175-177 °C. Fraction B24-B27, eluted with 15% ethyl acetate in hexane, was rotary-evaporated, furnishing a solid product. The compound was recrystallized from ethyl acetate/hexane mixture to give white needle-shaped crystals (0.5 g), m.p. 165-167°C, which was assigned as murrayanine (3). The crude chloroform extract from roots *M. koenigii* was fractionated by column chromatography using 5% ethyl acetate in hexane was obtained fraction D10 assigned as murrayafoline-A (4) (0.3 g).

A compound 4 was obtained as yellowish needle-shaped crystals and analyzed as  $C_{14}H_{13}NO$ . The IR spectrum of compound 4 showed a strong peak at 2996, 2942, 2916, and 2840  $cm^{-1}$  which were due to the stretching of C-H bonding from CH,  $CH_2$  or  $CH_3$  groups. The presence of C=C bonding was shown by strong peak at 1590  $cm^{-1}$ , where as aromatic ring absorptions were represented by peaks at 1502, 1450 and 1388  $cm^{-1}$ . The presence of NH peak occurs at 3448  $cm^{-1}$ . Peaks at 942, 852, and 742  $cm^{-1}$  correspond to substituted benzene ring. The EI-MS of compound 4 indicated the presence of molecular ion peak at  $m/z$  211 with the base peak of at  $m/z$  196.

The  $^1H$  NMR spectrum of this compound exhibited an H-bonded methoxyl group at  $\delta$  3.97. The coupling of the aromatic protons indicate that the ring A is ortho-substituted with two doublets at  $\delta$  8.01 and 7.37 attributed to H-5 and H-8, respectively. Another two pairs of triplets at  $\delta$  7.19 and 7.20 were due to H-6 and H-7, respectively. The  $^1H$  NMR also showed signals for one methyl group (H-9- Me) was present at  $\delta$  2.52, respectively. The other aromatic proton, H-4 appeared as singlet at  $\delta$  7.47.

The  $^{13}C$  NMR and DEPT spectrum indicated the presence of 12 carbons consisting quaternary carbons, aromatic carbons, and one methyl group and one methoxyl carbon. The methoxyl proton, which resonates at  $\delta$  3.97 showed cross peaks with C-1, and C-8a at 145.27 and 139.39 ppm. Other carbon signals include absorption peaks for geminal methyl carbons at 21.89 ppm aromatic carbon signals which appeared between 107 and 129 ppm. Further conformation of the structure was accomplished by analysis of HMQC, HMBC and COSY spectra (Table 1). All other compounds show the similar spectroscopic features with the carbazole alkaloids published in the literature.

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Table 1: NMR spectral data for compound 4 (murrayafoline-A)

Position	$\delta^1\text{H}$	$\delta^{13}\text{C}$	DEPT	HMQC correlations	HMBC correlations	COSY correlations
1	-	145.3	C	-	H-2, OMe	-
1a	-	123.5	C	-	H-6, H-8	-
2	6.72, <i>s</i>	107.6	CH	H-2	H-4, H-9	-
3	-	127.9	C	-	H-2, H-4, H-9	-
4	7.47, <i>s</i>	112.5	CH	H-4	H-2, H-6, H-9	-
4a	-	129.4	C	-	H-9	-
5	8.01, <i>d</i>	119.1	CH	H-5	H-7, H-8	H-6
5a	-	124.3	C	-	H-6	-
6	7.19, <i>t</i>	120.4	CH	H-6	H-7, N-H	H-5, H-7
7	7.20, <i>t</i>	110.9	CH	H-7	H-6	H-6, H-8
8	7.37, <i>t</i>	125.5	CH	H-8	H-5	H-7
8a	-	139.4	C	-	H-5, H-7	-
9-Me	2.52, <i>s</i>	21.9	CH <sub>3</sub>	H-9-Me	H-2, H-4	-
N-H	8.14	-	-	N-H	-	-
1-OMe	3.97, <i>s</i>	55.4	CH <sub>3</sub>	H-1-OMe	-	-

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